REMARKS

Concerning The Amendments

1. The specification

Applicants have amended the title to make it more descriptive of the claimed invention. Applicants have amended the application to reflect (a) that the United States government may have certain rights in the present invention, and (b) that grandparent application Serial No. 08/477,809 is now allowed.

Applicants have amended the specification to describe the present invention with greater particularity. Specifically, Applicants have amended the specification explicitly to recite that the present microarray invention includes microarrays having a density of at least about 400 positions per cm^2, corresponding to typical diameters of about 250 μm for regions in the microarray. Support for these amendments to the specification is found, among other places, in priority application Serial Nos. 08/261,833 (great-grandparent) and 08/477,809 (grandparent), which were incorporated by reference in the present application as filed at page 1, lines 4-15. For example, the great-grandparent application recites at page 8, lines 7-11, that each region in the microarray may be "about 25-**250** μm, and are separated from other regions in the array by about the same distance" (emphasis added). Similarly, the grandparent application recites at page 12, lines 5-9, that each region in the microarray may be "about 10-**250** μm, and are separated from other regions in the array by about the same distance" (emphasis added). It follows that a microarray having regions of 250 μm in diameter, spaced apart from one another by about the same distance (i.e., 250 μm) necessarily has 20 microarray positions per cm, which

is about 400 regions per cm^2. As discussed below, this density is recited in amended claim 7. These amendments do not constitute new matter.

2. The claims

Claims 1-6 have been canceled without prejudice, as being drawn to a non-elected invention. Applicants confirm their election, without traverse, to prosecute the invention of Group II in this application.

Claims 7, 8 and 17 have been amended to clarify what is claimed therein, as discussed below.

Amended claim 7 claims substrates comprising microarrays having a density of 400 or more regions per cm^2 and 400 or more regions. Support for these amendments is discussed above. Claim 7 also has been amended to recite a characteristic of Applicants' microarrays that is inherent in the method by which Applicants' microarrays are made. Specifically, claim 7 has been amended to recite that "each region in the microarray is essentially free of cross-contamination with DNA sequences applied to the other regions in the microarray." That limitation follows from the fact that Applicants' microarrays are created by individually depositing the desired DNA at each region in the microarray, necessarily precluding any significant degree of cross-contamination at any region in the microarray by DNA sequences applied to other regions in the microarray. *See generally* specification at 11-12, Serial No. 08/514,875 at 10-21, Serial No. 08/477,809 at 13-18, and Serial No. 08/261,388 at 8-14.

Claim 7 also has been amended to clarify that the polynucleotides at each region in the microarray are "isolated," meaning that they are not contained within cells. This limitation is

inherent in the description of Applicants' microarrays contained in this application, as well as in parent, grandparent and great-grandparent applications. As explained in the specification, the DNA sequences of Applicants' microarrays may be produced by a variety of methods including synthesis, amplification or excision from cells/chromosomes, etc., which methods all yield DNA sequences that are "isolated" in that they are not present in a cell. *See, e.g.,* specification at 9:25-35, 14:17-15:14.

Dependent claims 8 and 9 claim substrates comprising microarrays having, respectively, densities of 10,000 or more and 2,500 or more regions per cm^2. These densities are disclosed in the parent, grandparent and great-grandparent applications. For example, Table I of Serial No. 08/261,388 discloses microarray regions 50μm and 100μm in diameter, which corresponds--at equal interrgion distances--to 10,000 regions/cm^2 and 2,500 regions/cm^2, respectively. *See* Serial No. 08/261,388 at 8:10-11 and 11-12 (Table I).

Dependent claim 17 has been amended to clarify that the microarray may comprise polynucleotides that are fragments of mRNA-derived sequences and genomic DNA sequences.

Added dependent claims 18 and 19 specify that the microarray will comprise 2,500 or more regions (claim 18) and 10,000 or more regions (claim 19). These numbers are supported in this application, the parent application, the grandparent application and the great-grandparent application, as described above.

Added dependent claim 20 specifies that the DNA sequences are bound directly to the surface of the substrate.

Added independent claim 21 (and added claims 22-33, which depend therefrom) claims Applicants' microarray invention in a product-by-process format. Step (a) recites the step of depositing between about 0.002 nl and about 2 nl of a solution comprising a selected, isolated polynucleotide at a discrete region on the surface of the substrate bearing the microarray. That volume range is literally supported in this application (*e.g.*, at 12:5-6), the parent application (*e.g.*, at 14:2 and Table I), the grandparent application (at 16:34 and Table I), and the great-grandparent application (*e.g.*, at 11:31 and Table I). Step (b) recites that step (a) is repeated until a microarray of 400 or more regions is formed, at a density between about 62,500 and 625 regions/cm^2. The limitation of 400 or more regions is supported in this application and the parent, grandparent, and great-grandparent applications, as discussed above. The density range of between about 62,500 and 625 regions per cm^2 also is supported by all those applications. Those densities flow directly from the disclosure in all the applications that (1) the regions in the microarray may be formed by drops that are between about 0.002 nl and 2 nl, which correspond to disclosed region diameters of between about 20 and 200 μm, and (2) the distance between the regions in the microarray preferably should be separated from one another by about the same distance as the diameter of the regions.

Added dependent claims 22-33 correspond to claims 8-15 and 17-20.

Added independent claim 34 claims substrates "comprising a microarray of DNA sequences suitable for analysis of a polynucleotide mixture" that have densities of 400 or more regions/cm^2 and that comprise 400 or more discrete regions. Claim 35 further specifies that each of those 400 or more discrete regions contains "as an isolated polynucleotide, a characteristic

DNA sequence. . . ." Claim 34 also specifies that the microarray "comprises at least 400 regions essentially free of cross-contamination by DNA sequences characteristic of other of said 400 regions, such that the DNA sequences in said regions are selective in hybridizing with corresponding members of said mixture."

The value of 400 for density and number of microarray regions in claim 34 is fully supported, as described above.

By the term "a characteristic DNA sequence" in claim 34, Applicants mean that the DNA sequence constitutes the distinguishing feature of the subject region. *See* The Random House Dictionary of the English Language, 2d ed., unabridged (1987); *see also* specification at page 9, line 24. This limitation is inherent in the deposition process of Applicants' invention, as described in the specification. Specifically, as discussed above, in microarrays created by following Applicants' deposition process, each region is characterized by, and distinguished from other regions by, the presence of a single DNA sequence constituting under any conceivable circumstance) the vast majority of all polynucleotides present in the region. It should be noted that the claim 34 does not exclude substrates comprising regions whose characteristic DNA sequences are the same as others (e.g., controls), so long as the substrate comprises at least 400 regions whose respective characteristic DNA sequences are different, each from the other. Thus, for example, claim 34 as amended covers a substrate bearing 400 regions with characteristic DNA sequences that are distinct, each from the other, even if there are additionally present on the substrate any number of additional regions, e.g., serving as controls,

whose characteristic DNA sequences find counterparts in one or more of those in the "400 regions" referred to.

The term "selective in hybridizing" is supported, among other places, at page 14, lines 10-12 of the specification. The language in claim 34 regarding "cross-contamination" is fully supported, as described above.

None of the above amendments adds new matter.

As the densities in the pending claims now find literal support in Applicants' great-grandparent application Serial No. 08/261,388, the pending claims are entitled to the benefit under 35 U.S.C. § 120 of that application's July 17, 1994 filing date.

Issues Concerning 35 U.S.C. § 112

Claim 17 stands rejected under 35 U.S.C. § 112, second paragraph, because of the term "cDNA-derived sequences and genomic DNA sequences." Applicants have amended claim 17 to clarify that the claim also is intended to encompass fragments of those sequences. Claim 7, from which claims 8, 9 and 17 depend, specifies that the DNA sequences of the microarray are polynucleotides (which is defined at page 9, lines 5-7 of the specification). Thus, it is clear that the recited "cDNA-derived sequences, genomic DNA sequences and fragments thereof" of claim 17 must also be polynucleotides. Applicants respectfully submit that amended claim 17 is definite.

Issues Concerning 35 U.S.C. §§ 102 and 103

1. Heller

Claims 7-15 and 17 stand rejected under 35 U.S.C. § 102(e) as being anticipated by or, in the alternative under 35 U.S.C. § 103(a) as obvious over Heller et al. U.S. patent 5,605,662 ("Heller"). Heller refers to ordered arrays of biomolecules. Heller teaches that arrays are created by (1) providing a target region having a higher affinity for a biomolecule than surrounding regions, (2) flooding the entire array with a solution containing the first biomolecule intended for that higher-affinity region (relying on preferential binding to the higher affinity target region to localize the biomolecule), (3) removing the solution containing the first biomolecule, and (4) repeating the process for the next target region and next biomolecule.

Heller provides a target region having a higher affinity than surrounding regions for nucleic acid biomolecules by temporarily causing the target region to be positively biased, while the other regions are negatively biased. In theory, a negatively charged biomolecule, such as DNA, will preferentially bind to the positively biased target region.

A deficiency of the Heller method is that it relies on preferential binding to the target region. Because the entire substrate surface is flooded with the biomolecule, both the target and non-target regions necessarily will bind the biomolecule, albeit in differing amounts. This cross-contamination problem is magnified by Heller, because the entire surface is coated with an "attachment layer" having "optimal binding properties" (Heller at 13:29-37) for the applied biomolecule. Within practicable operating parameters, the differential biasing of target regions by Heller will not eliminate the finite affinity of applied biomolecules for the non-target regions:

the charge differential simply shifts the thermodynamic binding equilibrium from the non-target regions to the target regions.

Although Heller states that "[n]onspecific binding to negatively biased micro-locations will be negligible" (Heller at 30:26-28), Heller does not provide any quantitative data measuring the amount of cross-contamination that results from binding of biomolecules at regions other than the intended target region. Applicants are unsure what Heller means by "negligible" in that context. However, the prior art illuminates this issue. Heller's method of attaching DNA to the surface of the substrate is analogous to the method disclosed in Brown et al., Ultramicroscopy **38**:253-264 (1991) ("Brown") (copy attached hereto). In the Abstract, Brown states that "[a]lthough at no applied potential and at negative surface potentials some DNA was bound, at positive potentials 3 to 5 times more DNA was incorporated onto [graphite and gold] surfaces." While such differential binding may provide sufficient signal-to-noise ratios for many uses, the not-insignificant binding of any given biomolecule to non-target regions may constitute a liability for sensitive applications.

Another deficiency of the Heller arraying method is that it cannot be used to make arrays that both (1) contain a large number of regions, and (2) contain regions that are densely spaced. This is so because of the practical difficulties in providing the required circuitry for such arrays. The largest array exemplified by Heller contains 64 regions. At column 11, lines 58-63, Heller itself admits: "As the number of microlocations increases beyond several hundred, the complexity of the underlying circuitry of the micro-locations increases. In this case the microlocation grouping patterns have to be changed and spacing distances increased

proportionally, or multi-layer circuitry can be fabricated into the basic device." There is no teaching in Heller how to construct dense arrays of greater than several hundred regions. Further, the cross-contamination problem, discussed above, will only worsen as the number of regions in the array increases.

In contrast to Heller, independent claim 7 as amended (and dependent claims 8-20) are drawn to microarrays having 400 or more discrete regions of DNA sequences, at a density of about 400 or more regions per cm^2, where each region in the microarray is essentially free of cross-contamination with DNA sequences applied to other regions in the microarray. Dependent claims 8 and 9 recite even higher densities (10,000 and 2,500 per cm^2, respectively), while dependent claims 18 and 19 claim even larger microarrays (2,500 and 10,000 regions, respectively). The inventions of claims 7-20 are patentable over Heller for two independent reasons. First, as shown above, Heller does not enable (and teaches away from) the high-density, large microarrays of claims 7-20. Second, as shown above, Heller teaches away from microarrays where each region is essentially free of DNA sequences applied to other regions in the microarray. Such microarrays cannot be achieved using the Heller arraying method.

Added claim 21 (and dependent claims 22-31) are patentable over Heller for the same reasons as claims 7-20. Claim 21 is a product-by-process claim drawn to substrates comprising microarrays that are both high density (*i.e.*, between about 625 and 62,500 regions/cm^2) and large (*i.e.*, comprising 400 or more regions). As discussed above, such arrays are not taught or suggested by Heller. The claimed microarrays also are patentable in view of Heller, because the process steps recited in the claim require that each region in the microarray be created by

individually applying a selected DNA sequence to the desired region, resulting in microarrays that are distinctly different from those of Heller. Specifically, the microarrays of claim 21, because of their method of preparation, necessarily are characterized by regions that are essentially free of DNA sequences applied to other regions in the microarray. As discussed above, such arrays are not taught or suggested by Heller.

Added claim 34 (and dependent claim 35) are patentable over Heller for the same reasons as described above for claims 7-33.

In view of the foregoing, Applicants respectfully submit that the pending claims are neither obvious over nor anticipated by Heller.

2. Hozier

Claims 7-13 and 15-17 stand rejected under 35 U.S.C. § 103 as being obvious over Hozier U.S. patent 5,563,060 ("Hozier").

Hozier relates to microcolonies of cells (which comprise DNA sequences within them). All of the pending claims are now limited to microarrays of "isolated" DNA sequence. By "isolated" Applicants mean that the DNA sequences in the microarray are not contained within cells. No other limitation is intended by use of the term "isolated" in those claims. Applicants respectfully submit that this amendment overcomes the Section 103 rejection in view of Hozier.

Interview Summaries

Applicants' attorney appreciates the courtesy that was extended by the Examiner during the telephonic interviews on June 16, 1997 and November 17, 1997.

In the June 16, 1997 interview, the references Heller et al. (U.S. patent 5,605,662) and Barrett et al. (U.S. patent 5,252,743) were discussed generally as they related to the claims then pending. The Examiner stated his view that Heller et al. and Barrett et al. disclosed high density arrays of DNA oligomers long enough to be deemed "polynucleotides" -- a term then present in extant claims. The undersigned suggested that a written Office Action, by sharpening the issues, would facilitate Applicants' response.

In the November 17, 1997 interview, the undersigned stated that the Office Action mailed October 27, 1997 had not yet been received by her, and asked the Examiner to check the address to which that Office Action had been sent. The Examiner noted that a correspondence address change had not been entered as requested by Applicants, resulting in the Office Action being sent to the wrong address. The Examiner indicated that the Office Action would be re-sent, with a re-start of the response time, due to the previous incorrect addressing.

* * *

In view of the foregoing, entry of the amendments and allowance of the claims is respectfully requested. The Examiner is invited to contact the undersigned attorney at

(650) 614-4654 with any questions, comments or suggestions relating to the above-identified patent application.

Respectfully submitted,

Emily A. Evans

Emily A. Evans
Reg. No. 33,493

Attorney for Applicants

ARNOLD, WHITE & DURKEE
P.O. Box 4433
Houston, TX 77210
(650) 614-4500

Date: April 20, 1998